Validation of a Urine Assay to Measure Tenofovir Levels in Patients taking Tenofovir Alafenamide
IN-US-311-4388

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INTRODUCTION

Study Abstract

Pre-exposure prophylaxis (PrEP) with tenofovir/emtricitabine is 99% effective in preventing HIV when taken daily. PrEP is recommended in the U.S. by the CDC, and the World Health Organization globally, as a powerful tool for millions of individuals at risk for HIV. Adherence to PrEP is critical for prevention of new infections, but patient self-report and pill counts are unreliable methods for monitoring adherence. How to accurately identify suboptimal adherence, and develop strategic interventions to maintain adherence levels necessary for PrEP effectiveness in these populations, is the key gap in implementing this otherwise highly effective prevention therapy. Previously, we developed and validated a urine assay in patients taking **tenofovir disoproxil fumarate (TDF)**-based regimens, as this is the prodrug of tenofovir (TFV) that is currently approved in the form of tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) for PrEP in the United States. However, **tenofovir alefenamide (TAF)** is replacing TDF as an equally effective and less toxic tenofovir prodrug in HIV treatment regimens, i.e. TAF/FTC/EVG/COBI, TAF/RPV/FTC, and TAF/FTC. As we scale up use of this urine assay, it is critical to ensure that this urine assay remains valid for detecting TFV in patients taking TAF-based regimens. We propose to validate the assay in three separate populations: 1a) ten HIV-negative subjects who are given seven directly observed daily doses of TAF/FTC and then tested daily for ten days to assess the half-life of tenofovir in urine in order to determine the length of time TFV can be measured in the urine after last dose is taken in the context of consistent adherence; 1b) ten HIV-negative subjects who are given one directly observed dose of TAF/FTC and then tested daily for seven days to assess the half-life of tenofovir in urine in order to determine the length of time TFV can be measured in the urine after one dose; and 2) ten HIV-positive subjects with undetectable viral loads on TAF-containing regimens will have a one-time urine and plasma collection prior to their daily dose to determine urine TFV concentration in the setting of steady state dosing.

Primary Hypotheses

Aim 1) To determine how long TFV is excreted in the urine in patients who have taken seven daily doses (cohort 1a) or one dose (cohort 1b) of TAF/FTC. This will allow us to assess the length of time TFV can be measured in the urine after last dose is taken (the “lookback” period) in the context of steady state/consistent dosing (cohort 1a), or in the context of inconsistent or intermittent adherence (cohort 1b) as well as to determine how many days a patient has been off drug if a urine specimen has no detectable TFV. Based on the pharmacokinetics of TAF, we hypothesize that comparable cut-offs indicative of no/intermittent/recent adherence in patients on TAF-based regimens will be approximately 1 log (or 10-fold) lower than in patients taking TDF-based regimens. Accordingly, we hypothesize the TFV concentration in urine associated with protective levels in plasma for patients on TAF-based regimens will be 100 ng/mL instead of 1000 ng/mL (i.e. 1 log lower). We also hypothesize that TFV will be detectable in the urine 1-2 days longer in cohort 1a than in cohort 1b, and knowledge of these nuances in detection will allow for a more robust interpretation of urine testing in the clinic setting.

Aim 2) To determine the expected urine tenofovir levels in a population of HIV-positive patients who are virologically suppressed on TAF-based HIV treatment regimens. Urine and plasma TFV values will be compared to a historical cohort of patients on TDF-based regimens. Since TAF concentrates more highly in the intracellular space and less in the plasma space, resulting in approximately 10% of the plasma TFV concentrations compared to TDF, we anticipate that both plasma and urine TFV concentrations will likewise be approximately 10% of the values in TDF patients (i.e. 1 log lower) assessed under the same conditions.

Purpose of Study Protocol

Tenofovir alefenamide (TAF) is replacing TDF as an equally effective and less toxic TFV prodrug in HIV treatment regimens, i.e. TAF/FTC/EVG/COBI and TAF/RPV/FTC, and it may ultimately be utilized for HIV prevention given promising animal data. Our underlying hypothesis is that the urine assay will accurately detect urine TFV levels in patients on TAF-based regimens, although measured concentrations may be lower in patients taking TAF/FTC compared to those taking TDF/FTC. Given that administration of TAF results in plasma TFV concentrations 1 log lower than in patients given TDF, we expect that urine TFV concentrations will also be 10-fold lower. Accordingly, we hypothesize the TFV concentration in urine associated with protective levels in plasma for patients on TAF-based regimens will be 100 ng/mL instead of 1000 ng/mL (i.e. 1

log lower). Using our current methodology, where the lower limit of detection (LOD) is 5 ng/mL, the assay should easily have the capability to detect TFV in urine of patients taking TAF. Detection of urine TFV in patients taking TAF-based regimens has the potential for use in clinical trials using TAF/FTC for PrEP and in the clinical setting for ongoing adherence monitoring should TAF/FTC be approved for PrEP. We hypothesize that TFV will be present in the urine of subjects for 3-5 days after receiving one dose of TAF/FTC and 5-7 days in subjects given 7 daily doses of TAF/FTC, and will decay in a log-linear fashion allowing for determination of a concentration "cut-off" indicative of recent (last 1-2 days) adherence. We also hypothesize that TFV will be detectable in urine in all or most patients in cohort 2, as subjects are presumably adherent HIV-positive patients on TAF-based regimens, and that urine TFV concentrations will be approximately 1 log (10-fold) lower than those in historical patients taking TDF-based regimens.

BACKGROUND

Prior Literature and Studies

TDF/FTC reduces HIV transmission in individuals at risk of infection. Several studies have demonstrated the utility of TDF/FTC preexposure prophylaxis (PrEP) in preventing the acquisition of HIV infection (1-3). The CDC and WHO recommend PrEP for groups at risk of infection including men who have sex with men (MSM), HIV-negative partners of HIV-positive individuals, intravenous drug users, and certain heterosexual men and women (4)**.** PrEP is a once daily tablet that blocks productive infection (5). TDF/FTC is at the present time the only drug approved for this indication and is tolerable and safe. TDF/FTC provides high drug concentrations in the male and female genital tracts, and decreases the risk of transmission or development of resistant virus by acting early in the HIV life cycle through the synergistic effect of the two drug components (1, 5-9).

Adherence to PrEP correlates closely with efficacy, but current measurements of adherence are inadequate. The iPrEX trial demonstrated that 2499 MSM who took TDF/FTC had a 44% reduction in HIV acquisition overall compared to those who took placebo, vs a 99% reduction in those who took PrEP daily (1, 10). Directly observed dosing in the STRAND study yielded intracellular tenofovir concentrations that corresponded with HIV-1 risk reduction of 76% with 2 doses per week, 96% with 4 doses per week, and 99% with 7 doses per week (10).The importance of adherence was also demonstrated in the TDF2 trial of heterosexual men and women in which efficacy of TDF/FTC was 63% overall and 78% in consistent users (3), and in the Partners PrEP trial in which efficacy was 73% overall and 90% in consistent users (2). In contrast, TDF/FTC was found to be ineffective in preventing HIV infection in the Fem-PrEP (11) and VOICE trials, in which adherence was demonstrated to be poor. Self-reported adherence and pharmacy refill data alone do not correlate well with actual adherence in PrEP trials (12). **In yMSMc in an urban setting, rates of detectable plasma tenofovir concentrations ranged from 63.2% at week 4 to 20% at week 24 despite high levels of self-reported adherence to PrEP** (13)**.** The same was true of trials looking at women such as Fem-PrEP: less than 40% of a representative sample of subjects had detectable drug in plasma consistent with therapeutic dosing, despite 95% of women reporting that they "always" or "usually" used the product, and pill counts suggesting that study drug was taken on 88% of days (11, 14).

Antiretroviral concentrations in urine are potentially useful in monitoring adherence to PrEP. Although clinical data are limited, lamivudine concentrations in urine have been used as a means of monitoring antiretroviral adherence. Due to its short half-life of 5 to 7 hours, lamivudine was largely absent from the urine 24 hours after a single dose; thus a lamivudine concentration of 0.035 mg/mg creatinine or less at 48 hours was suggestive of a missed dose the previous day (15). Tenofovir (TFV) is the active metabolite of the prodrug tenofovir disoproxil fumarate (TDF), and is attractive for monitoring adherence as it has a plasma half-life of 17 hours and intracellular half-life of 150 hours (16), which allows detection in urine for several days. In patients taking TDF-based regimens, we have shown that TFV concentrations can be reliably measured in urine, that urine TFV concentrations correlate well with plasma concentrations, and TFV detection in urine reflects medication usage over a window of 1 to at least 7 days after oral TDF/FTC ingestion.

Therapeutic drug monitoring (TDM) has been effective in monitoring and maintaining drug concentrations within the therapeutic window in other clinical settings. TDM is useful in identifying inadequate adherence as a cause of poor treatment response in a variety of fields (17-20), including psychiatric medications in outpatients with co-morbid substance abuse disorders (17), treatment of substance

abuse disorders for certain reference drugs including bupropion, buprenorphine, disulfiram, methadone, and naltrexone (18), and improving efficacy and reducing toxicity of several antiepileptic medications (21). In patients with refractory hypertension, a large study found that when patients were informed of their undetectable drug levels and provided additional counseling, blood pressure control was markedly improved without increasing treatment intensity (20). While it is possible that patients may be concerned that urine specimens may also be used to detect illicit drugs, to our knowledge none of these studies have raised that concern and that has not been our experience now in over 160 individuals in which we have used this assay in the field. Additional limitations to TDM mentioned in the literature include "white coat compliance" (improved adherence preceding a clinic visit) that may limit the ability to rely completely on results of TDM (22), and the concern that TDM may not be appropriate for all clinical settings in its current form. However, a tool for objective adherence monitoring that is non-invasive and easy to incorporate into a clinical setting would be a powerful adjunct to standard of care clinical adherence support.

Urine Testing is Preferable to other Objective Adherence Measurements for PrEP. Although other PK-based measures of adherence are being studied in ongoing trials, the CDC and other groups believe that urine may be the best matrix in which to measure adherence to PrEP. The ultimate effectiveness of a **urine-based screen** for adherence and its usefulness in the clinical setting derive from three innovative aspects of this assay and from our study design.

1. Noninvasiveness. Urine TFV assessment is preliminarily highly acceptable to yMSMc (23). Adolescents demonstrate a significant preference for *non-blood draw or needle-requiring assays* in HIV testing (50.5% chose rapid oral swab vs. 30.3% traditional venipuncture vs. 19.2% rapid finger stick blood test) and are likely to prefer urine collection to methods that require needles especially in the setting of frequent monitoring. Especially among young MSM, the most at-risk for HIV acquisition, urine collection provides advantages over blood draw for plasma or intracellular concentration, finger prick for DBS, and pubic/scalp hair sampling.

2. Specific window period of urine TFV assay. Urine TFV assessment fills a gap left by plasma, DBS, and hair assessments by providing information about medication adherence over at least a one-week period: single plasma concentrations only reflect a small window of exposure (2-3d) (24-26), and hair analysis and DBS reflect average drug exposures over 1-3 months (27, 28). In yMSMc, current (previous week) adherence data may have greater value than average adherence over the prior 3 months given increased vulnerability to HIV exposure, and may create a greater number of opportunities for clinicians to reinforce PrEP adherence behaviors.

3. A urine assay lends itself well to development of a point-of-care (POC) assay, and we have received CFAR pilot funding to collaborate with the Physics Department at the Univ. of Penn. to develop a grapheme-aptamer biosensor POC device that detects TFV in urine, as well as a phase I small business innovation grant through the NIH to develop a lateral flow point-of-care device. Our goal is to make this methodology widely available in a variety of clinical and non-clinical settings. This study represents an opportunity to assess the ability of urine testing to improve adherence in order to inform ongoing efforts for POC development.

Tenofovir alafenamide (TAF) is a novel prodrug of TDF that has been approved in combination with emtricitabine as an agent for HIV treatment. When compared to standard dose TDF (300 mg/daily), TAF, at a dose of 25 mg/day, has a 7-fold higher peripheral blood mononuclear cell intracellular tenofovir diphosphate concentration, with approximately 10% of the plasma tenofovir exposure. That is, patients taking TAF will have about 90% less TFV in the plasma than patients taking TDF, which results in decreased systemic toxicity most importantly on bones and kidneys. At steady state, 25 mg of TAF yielded mean TFV plasma exposures [area under the plasma concentration-time curve (AUCtau)] of 86% lower as compared with the TFV exposures observed with 300 mg of TDF. This may translate into greater antiviral efficacy, a higher barrier to resistance, and an improved safety profile relative to TDF (29). In an analysis of two double-blind trials in subjects who were treatment-naïve, 92% of subjects on elvitegravir/cobicistat/emtricitabine/TAF achieved an HIV RNA <50 copies/ml at 48 weeks compared to 90% of subjects on elvitegravir/cobicistat/emtricitabine/TDF, demonstrating non-inferiority (30). Smaller decreases in eGFR and bone mineral density of the hip and spine were also seen in those receiving TAF compared to TDF (30). This TAF-based medication was approved in November 2015. A second TAF-based regimen, TAF/rilpivirine/emtricitabine, was approved in February 2016, and TAF/FTC was approved for use with another antiretroviral agent or agents in April 2016.

Preliminary Data

Urine Assay Development & Validation We developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) urine assay with high sensitivity and specificity for TFV. This assay determines TFV concentrations in log categories from 0 ng/mL to > 10,000 ng/mL (23, 33, 34).
1. ***Urine TFV concentration was predictive of when last dose of TDF/FTC was taken.*** TFV was detected for >7 days in urine and cleared in a log-linear fashion, with a direct correlation to time since last dose.
2. ***The urine assay distinguished between recent adherence (within 48 hours), low adherence (within 1 week but not within the previous 48 hours), and non-adherence to PrEP.*** In a 24-week study of 10 HIV-negative subjects receiving daily PrEP, urine TFV concentration > 1000 ng/mL was ***highly predictive*** of presence of TFV in plasma (>10 ng/mL) (PPV 0.95, 95%CI, 0.82-0.99; NPV 0.79, 95%CI, 0.49-0.95), suggesting that the urine assay could distinguish between recent adherence (>1000 ng/mL), low adherence (>10 to >100 ng/mL), and non-adherence as defined by last dose more than one week prior (0 ng/mL). Sensitivity and specificity were calculated over a variety of cut-points, and 1000 ng/mL was selected due to both high sensitivity (0.92, 95%CI, 0.79-0.98) and specificity (0.85, 95%CI, 0.55-0.98).
3. ***A prospective study of 50 PrEP patients at FIGHT was conducted for 48 weeks using urine TFV measurements to monitor adherence to PrEP.*** In this study, urine TFV testing provided clinically important information regarding adherence over a 48-week period, and was highly acceptable to participants. Clinical records were flagged for those in whom urine testing demonstrated no or inconsistent adherence to PrEP. This information allowed clinicians to immediately respond and intervene focusing on individuals at higher risk of acquisition of HIV.
4. ***Urine TFV testing was shown to be highly correlated with the research gold standard for objective adherence monitoring, dried blood spot (DBS).*** Urine TFV levels >1000ng/mL demonstrated sensitivity of 94% (95% CI: 88-99), PPV of 95% (95% CI: 90-100), specificity of 56% (95% CI: 23-88) and NPV of 50% (95% CI: 19-81) for ≥700 fmol/punch. Urine TFV levels >1000ng/mL had sensitivity of 98% (95% CI: 95-101), PPV of 69% (95% CI: 59-79), specificity of 26% (95% CI: 12-41), and NPV of 90% (95% CI: 71-109) for ≥1250 fmol/punch. Urine TFV >1000ng/mL had a sensitivity of 99% (95% CI: 96-100) and PPV was 95% (95% CI: 90-101) for detectable DBS FTC-TP. Urine TFV specificity and NPV were 69% (95% CI: 44-94) and 90% (95% CI: 71-109) for DBS FTC-TP.

Rationale for the Study

TAF/FTC is currently being evaluated for use as PrEP. Data presented at CROI 2016 demonstrated that tissue penetration of TAF in the genital and rectal tissue of healthy women were lower than expected, with TFV concentrations 2-10 fold lower in genital and rectal tissue than with TDF, and TFV-DP exposure was 13-fold lower in rectal tissue and 1-fold lower in the female genital tract compared to TDF, even though levels in plasma and cells were consistent with prior studies (31). However, animal data suggest that macaques given daily TAF at a human-equivalent dose are protected from rectal simian HIV (SHIV) (32). A large trial is ongoing by Gilead Sciences that will study TAF/FTC compared to TDF/FTC for this purpose to provide a definitive answer to this critical question. Both TDF and TAF are prodrugs of tenofovir (TFV), which is the molecule detected by the urine assay; thus we hypothesize that the urine assay will also be able to assess adherence in patients taking TAF-based regimens. The purpose of this study is to formally re-validate the urine TFV assay in patients taking TAF-based regimens.

STUDY OBJECTIVES

Primary Objectives:
1a) To determine how long TFV is excreted in the urine in patients at steady state of TAF/FTC. Ten healthy subjects will be given seven daily doses of TAF/FTC under direct observation to ensure adherence. Morning urine and plasma samples will be collected starting the day the last is given (1 hour later) and every day thereafter for 9 days (total of 10 days of sample collection). This will allow us to assess the length of time TFV can be measured in the urine after last dose is taken (the “lookback” period) in the context of consistent adherence, as well as to determine how many days a patient has been off drug if a urine specimen has no detectable TFV. A correction analysis similar to that above will also be assessed in this cohort.

1b) To determine how long TFV is excreted in the urine in patients who have taken one dose of TAF/FTC. Ten healthy subjects will be given one dose of TAF/FTC under direct observation to ensure adherence. Morning

urine and plasma samples will be collected starting the day the dose is given (1 hour later) and every day thereafter for 6 days (total of 7 days of sample collection). This will allow us to assess the length of time TFV can be measured in the urine after last dose is taken (the "lookback" period) in the context of inconsistent or intermittent (1 day only) adherence, as well as to determine how many days a patient has been off drug if a urine specimen has no detectable TFV. We will also look at the best way to correct urine TFV values for inter-subject variability by assessing which measure (specific gravity, urine creatinine, pH) will maximize the correlation between urine TFV levels and an ideal line of elimination.

Secondary Objective:
To determine the expected urine tenofovir levels in a population of HIV-positive patients on TAF-based regimens. A cross-sectional analysis of ten HIV-positive patients with undetectable viral loads on a TAF-based single tablet HIV regimen will be conducted. Morning urine and plasma samples will be collected at one time point to determine urine TFV concentration in the setting of steady state dosing in HIV patients with presumably very good adherence to medication, and compared to a historical cohort of patients on TDF-based regimens.

Rationale for the Selection of Outcome Measures

Cohort 1a and 1b
For urine tenofovir testing to represent a useful test, clinicians, counselors and public health practitioners need to be able to interpret a negative urine tenofovir result accurately; i.e. in the setting of a negative urine test, for how many days prior to the urine test has the patient likely been nonadherent to medication? In our previous data in 10 healthy subjects given one dose of TDF/FTC, we found that TFV was detected for >7 days in urine and 2-3 days in plasma after a single dose of TDF/FTC, and that urine TFV concentration was predictive of when the last dose of TDF/FTC was taken. Based on these data, we were able to predict if a subject has taken TDF/FTC in the last 1-3 days if concentrations were > 1000 ng/mL, or not at all within the last week if concentrations were < 10 ng/mL. This aim is conducted to determine the time course of urine and plasma tenofovir decay in subjects given TAF/FTC, and to compare these data to historical data in subjects given TDF/FTC. Based on the pharmacokinetics of TAF, we hypothesize that comparable cut-offs indicative of no/intermittent/recent adherence in patients on TAF-based regimens will be approximately 1 log (or 10-fold) lower. Accordingly, we hypothesize the TFV concentration in urine associated with protective levels in plasma for patients on TAF-based regimens will be 100 ng/mL instead of 1000 ng/mL (i.e. 1 log lower). Using our current methodology, where the lower limit of detection (LOD) is 5 ng/mL, the assay should easily have the capability to detect TFV in urine of patients taking TAF. We will look at the time course of TFV decay in the urine in subjects given <u>seven</u> daily doses of TAF/FTC (Aim 1a) and in subjects given <u>one</u> dose of TAF/FTC (Aim 1b) to assess the differences in patients with different adherence patterns. Seven days of daily dosing has been recommended as the minimum number of daily doses required to be protected from HIV infection (at least from rectal exposure to HIV) and thus we chose this number of doses in Aim 1a. We hypothesize that TFV will be detectable in the urine 1-2 days longer in the former cohort, and knowledge of these nuances in detection will allow for a more robust interpretation of urine testing in the clinic setting.

Cohort 2
We would like to determine how urine TFV concentrations in HIV-positive subjects taking a TAF-based regimen compare to those taking TDF-based regimens. We previously showed in ten HIV patients taking daily TDF-based antiretroviral therapy regimens that detection of urine TFV corresponds to plasma TFV concentrations within a 24 hour dosing window. Ten HIV-positive patients with high adherence to a single-tablet HIV regimen containing TDF (as measured by having an undetectable viral load within 4 weeks of study testing) were asked to provide a single plasma and urine sample within 12 to 24 hours of their last dose of medication. This study demonstrated 100% concordance between presence of TFV in plasma and urine (PPV 100%, 95% CI, 0.63-1.0; NPV 100%, 95%CI, 0.05-1.0). TFV concentration was 3-4 logs higher in urine than plasma. Subject 4 had an undetectable viral load within 4 weeks of sample collection but reported later that he had stopped taking his antiretroviral therapy shortly after his viral load had been collected; TFV was detected in neither plasma nor urine in this subject. Since TAF concentrates more highly in the intracellular space and less in the plasma space, resulting in approximately 10% of the plasma TFV concentrations compared to TDF, we anticipate that both plasma and urine TFV concentrations will likewise be approximately 10% of the values in

TDF patients (i.e. 1 log lower) assessed under the same conditions.

STUDY DESIGN AND METHODOLOGY

Design Summary

1a) Ten healthy subjects will be given seven daily doses of TAF/FTC under direct observation to ensure adherence. Morning (not first morning) urine and plasma samples will be collected starting the last day the dose is given (1 hour post-dose) and every day thereafter for 9 days. This will allow us to assess the length of time TFV can be measured in the urine after last dose is taken (the "lookback" period) in the context of consistent adherence, as well as to determine how many days a patient has been off drug if a urine specimen has no detectable TFV.

1b) Ten healthy subjects will be given one daily dose of TAF/FTC under direct observation to ensure adherence. Morning (not first morning) urine and plasma samples will be collected starting the day the dose is given (1 hr post-dose) and every day thereafter for 6 days. This will allow us to assess the length of time TFV can be measured in the urine after last dose is taken (the "lookback" period) in the context of consistent adherence, as well as to determine how many days a patient has been off drug if a urine specimen has no detectable TFV.

2) Ten HIV-positive patients who have had undetectable viral loads for greater than 12 weeks (and a recent undetectable viral load in the previous 4 weeks) on an antiretroviral regimen containing TAF/FTC (i.e. Genvoya™, Odefsey™, or Descovy™ in combination with another HIV medication or medications) will have one-time pre-dose urine (early morning) and plasma samples drawn for tenofovir (TFV) concentration, as well as a comprehensive metabolic panel for measurement of creatinine clearance.

Study Setting

Recruitment/enrollment and study procedures will be carried out at Philadelphia FIGHT, a community-based federally qualified health center. At FIGHT, we have a clinical PrEP program focused on yMSMc and transgender women that has been nationally recognized for success in engaging and retaining high-risk youth in PrEP care. Housed within Y-HEP is a research team that conducts PrEP-related research with a subset of patients taking PrEP, which allows for dedication of research staff to this current proposal. Urine and plasma samples will be shipped to the Children's Hospital of Philadelphia (CHOP) Pharmacology Research Unit for tenofovir quantification. The Hospital of the University of Pennsylvania, affiliated with FIGHT through clinical and research collaborations, will provide mentoring and statistical support through the Penn Center for AIDS Research (CFAR).

Study schema and schedule of events:
Cohort 1a

	Screening visit	Dose of TAF/FTC	Dose of TAF/FTC									
DAYS	**Up to -14**	**0 to 5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
HIV Ag/Ab test	**X**											
CMP	**X**											
Hep B SAg/SAb	**X**											
Pregnancy test	**X**											
Urine TFV			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Plasma TFV			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

Urine studies			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

Note: HIV Ag/Ab test = human immunodeficiency virus antigen/antibody test (4th generation); CMP = comprehensive metabolic panel; Hep B SAb/SAg = hepatitis B surface antibody/surface antigen; TFV = tenofovir; urine studies = urinalysis, specific gravity, creatinine concentration); TAF/FTC = tenofovir alefenamide/emtricitabine

Cohort 1b

	Screening visit	**Dose of TAF/FTC**						
DAYS	**Up to -14**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
HIV Ag/Ab test	**X**							
CMP	**X**							
Hep B SAg/SAb	**X**							
Pregnancy test	**X**							
Urine TFV		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Plasma TFV		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Urine studies		**X**	**X**	**X**	**X**	**X**	**X**	**X**

Note: HIV Ag/Ab test = human immunodeficiency virus antigen/antibody test (4th generation); CMP = comprehensive metabolic panel; Hep B SAb/SAg = hepatitis B surface antibody/surface antigen; TFV = tenofovir; urine studies = urinalysis, specific gravity, creatinine concentration); TAF/FTC = tenofovir alefenamide/emtricitabine

Cohort 2

	Screening visit	**Study visit**
DAYS	**Up to -30**	**0**
HIV viral load	**X**	
Urine TFV level		**X**
Plasma TFV level		**X**
Urine studies		**X**

Note: HIV viral load = human immunodeficiency viral load; TFV = tenofovir

Subject Selection and Withdrawal

Inclusion and Exclusion Criteria

Cohort 1(a & b) Inclusion Criteria:

- Age 18 or older at the time of signed informed consent
- Not currently taking commercial Truvada for PrEP or any other investigational, oral medication for the purpose of HIV PrEP
- Willing and able to independently provide written informed consent
- Tests HIV negative at time of screening using rapid HIV antibody test or serum antibody/antigen 4th generation HIV test

Cohort 1(a & b) Exclusion Criteria:

- Evidence of acute or chronic hepatitis B infection at the time of screening
- Other clinically significant acute or chronic medical condition, including severe infections requiring treatment such as tuberculosis, as determined by the study investigator
- Evidence of renal dysfunction (Creatinine Clearance < 30 ml/min) at the time of screening; Use Cockroft-Gault equation: GFR = (140-Age in years) x (Weight in kg) / (72 x serum creatinine)

- History of bone fractures not explained by trauma
- Grade 3 laboratory abnormality on screening tests/assessments as defined by the DAIDS grading system
- Known allergy/sensitivity to the study drug or its components
- Experiencing decompensated cirrhosis (e.g., ascites, encephalopathy, etc.)
- Any other clinical condition or prior therapy that, in the opinion of the Principal Investigator, would make the subject unsuitable for the study or unable to comply with the dosing requirements

Cohort 2 Inclusion Criteria:
- Age 18 or older at the time of signed informed consent
- Willing and able to independently provide written informed consent
- Last viral load < 20 copies/mL within the last four weeks of screening
- Must be on combination antiretroviral therapy that includes TAF/FTC for at least 6 months
- Undetectable viral load, as defined by < 50 copies/ml, for at least 6 months

Cohort 2 Exclusion Criteria:
- Other clinically significant acute or chronic medical condition, including severe infections requiring treatment such as tuberculosis, as determined by the study investigator
- Evidence of renal dysfunction (Creatinine Clearance < 30 ml/min) at the time of screening; Use Cockroft-Gault equation: GFR = (140-Age in years) x (Weight in kg) / (72 x serum creatinine)
- Grade 3 laboratory abnormality on screening tests/assessments as defined by the DAIDS grading system
- Experiencing decompensated cirrhosis (e.g., ascites, encephalopathy, etc.)
- Any other clinical condition or prior therapy that, in the opinion of the Principal Investigator, would make the subject unsuitable for the study or unable to comply with the dosing requirements

Ethical Considerations

The proposed studies will only enroll adults ages 18 years and older. No exclusion will be made with respect to race or sex.

Subject Recruitment Plans and Consent Process

The study will be conducted at Philadelphia FIGHT in accordance with the standards of care and confidentiality provided to all clients.

Subjects will be recruited through word of mouth, flyers, social networking sites such as Facebook and smart phone applications such as Grindr, advertising, and by networking with other organizations such as the CHOICE hotline in Pennsylvania. The enrollment period for each cohort (cohort 1a, 1b, and 2 will be separate cohorts) will last for a maximum of 3 months, and will be rolling to allow for entry into the program as soon as possible for enrolled individuals.

All subjects who are interested in participating in the study will be screened according to the inclusion and exclusion criteria. To document consent, the subject will sign an Institutional Review Board (IRB)-approved informed consent, which will be specific for this study and in accordance with Good Clinical Practice (GCP) guidelines. The informed consent is written in a language that the subject can understand. The consent process will be conducted by the Principal Investigator or by adequately trained research staff prior to any study related procedures taking place. Once informed consent is given, all study subjects will be assigned a patient number.

Risks to the Subjects

There will be limited risks in this study. TAF/FTC is FDA-approved for HIV treatment in conjunction with other medications, and is known to be a safe and well-tolerated medication. TAF/FTC, while not approved for HIV prevention, is currently under study in a 5000-subject trial in a healthy HIV-negative population. Additional study procedures outside of study drug administration are limited to collection of plasma and urine specimens.

Protection Against Risks

There should be minimal risks associated with this study. Philadelphia FIGHT has a long history as a research agency. There is a research coordinator who will directly oversee the study research assistants in recruitment, safety, and monitoring of subjects. There is a full-time clinic at Y-HEP staffed by nurse practitioners and a 24-hour clinician on call for Philadelphia FIGHT should any participant suffer an adverse event during the course of this trial. Subjects will receive general health care and study-specific care regardless of insurance status throughout the course of this study. The phone number for participants to call will be listed on the informed consent and provided to them on several occasions. Subjects will be monitored closely for side effects during treatment with TAF/FTC, should they occur. Any participant who becomes positive for HIV during this study will discontinue TAF/FTC and will be linked to care immediately upon diagnosis at the Lax Center or another HIV clinic of their choice.

Subjects in cohorts 1a and 1b will be instructed at the screening visit and throughout the study that TAF/FTC should not be expected to provide protection against HIV as it has not yet been shown to be effective in this capacity. Other preventive options (condoms, risk reduction techniques) will be reviewed and offered during the study, and subjects interested in PrEP will be offered a referral to FIGHT's PrEP program upon completion of the study.

Study Drug

General Information

Tenofovir alafenamide/emtricitabine (TAF/FTC) is used in combination with other antiviral medications to treat HIV. It is not currently approved by the FDA as monotherapy for HIV prevention. TAF/FTC works by slowing the spread of HIV in the body for those who are infected, and is currently being tested in a large-scale trial to see if it is effective in preventing infection for those who are HIV-negative. TAF/FTC is not a cure and may not decrease the number of HIV-related illnesses.

TAF/FTC comes as a tablet to take by mouth and should be taken as indicated in the study design for all three cohorts.

Side Effects

TAF/FTC may cause side effects. When used alone, or in combination with other antiviral medications, TAF/FTC may cause serious damage to the liver and a condition called lactic acidosis. Potential study subjects should notify the Study Doctor if they drink large amounts of alcohol and if they currently have or ever have had liver disease.

The study doctor should also be notified if the patient experiences any of the following symptoms: upset stomach, loss of appetite, excessive tiredness, weakness, dark yellow or brown urine, unusual bleeding or bruising, flu-like symptoms, yellowing of the skin or eyes, and pain in the upper right part of your stomach. Other possible symptoms include: diarrhea, vomiting, and gas.

Treatment Regimen

- Cohort 1 – Cohort 1 will be administered TAF/FTC as per study protocol.

- Cohort 2 – Cohort 2 will continue to receive their standard-of-care dosage of anti-retroviral medication(s) and will therefore experience no change to their treatment regimen.

Preparation and Administration of Study Drug

- Cohort 1 – Study subjects will take the drug at the dose that is currently under study for HIV prevention. TAF/FTC will be supplied through Gilead through this research grant and administrated to subjects in Cohort 1 by delegated site study staff.
- Cohort 2 – Cohort 2 will already be taking the study drug in the correct dose (the dose taken for treatment is the same dose under study for prevention) and the study subjects will continue to supply and administer the drug as per the standard of care for HIV treatment.

Subject Compliance Monitoring

- Cohort 1 – Medication compliance will be measured through directly observed administration.
- Cohort 2 – Medication adherence will be optimized through ensuring patients meet inclusion criteria, specifically those that require an undetectable viral load in the last 4 weeks and undetectable viral load for at least 6 months. In addition, subjects in cohort 2 will be asked to record in a diary the time the medication was taken the day before study visit 1 when samples are drawn.

Prior and Concomitant Therapy

- Cohort 1 – Cohort 1, according to inclusion and exclusion criteria, will not be on antiretroviral therapy, and ideally will not be on any other medication.
- Cohort 2 – Cohort 2 will continue to receive combined antiretroviral therapy in accordance with the standard of care for HIV treatment.

Receiving, Storage, Dispensing, and Return

TAF/FTC will be dispensed by Gilead, Inc., stored at FIGHT in a secure location, and provided to the subjects by the study coordinator or designated study staff.

- Cohort 1 – For Cohorts 1a and b, medication will be obtained by study staff from the manufacturer through this study, stored on site as per package insert instructions, and dispensed by delegated study staff at Philadelphia FIGHT.
- Cohort 2 – Cohort 2 will already be taking the study drug and will continue to manage the reception, storage, and dispensing of their medication.

STUDY PROCEDURES

Screening for Eligibility

After patients have been informed about the trial and found to meet all inclusion criteria, written informed consent – in accordance with good clinical practice (GCP) and the Philadelphia FIGHT IRB – must be obtained prior to any study related procedures taking place. Once informed consent is given, all study subjects will be assigned a patient number.

The screening visit should take place no more than 14 days before Visit 1 for cohort 1 and 30 days for cohort 2; however, the interval between the screening visit and Visit 1 may be extended at the discretion of the study

team. Patients who have a laboratory test value outside the range specified by the inclusion criteria may have the test repeated once to determine eligibility; however, the result must be available prior to Visit 1.

Participants will be consented and baseline demographics (age, race, sex), HIV status and HIV treatment regimen, when applicable, will be collected. Laboratory testing will be assessed during the screening visit as above:

Cohort 1 – At the screening visit, subjects will be asked to review and sign the informed consent once all questions have been answered. Medical history and other concurrent medications will be documented. Vital signs (blood pressure, temperature, respiratory rate, and heart rate) will be taken. Blood will be drawn for comprehensive metabolic panel, hepatitis B SAb, hepatitis B SAg, and serum pregnancy test (for women). HIV testing at the time of screening will be done either by blood test or rapid testing (fingerstick or oral swab using an FDA-approved testing kit).

Cohort 2 – At the screening visit, subjects will be asked to review and sign the informed consent once all questions have been answered. Medical history and other concurrent medications will be documented. Vital signs (blood pressure, temperature, respiratory rate, and heart rate) will be taken. A blood sample will be drawn for HIV viral load if this has not been documented in the medical chart in the last 4 weeks. A diary will be provided to study subjects with specific instructions on which date to return for the first study visit, and instructions to record the time they take their medication on the day prior to the study visit.

Schedule of Measurements

After screening, subjects in Cohort 1a will have seven daily study visits for directly observed medication (last visit will also have samples collected), and nine daily study visits for urine and plasma sample collection. Subjects in Cohort 1b will have one visit for directly observed medication and plasma and urine collection, and six daily study visits during which they will have daily blood and urine samples collected. Subjects in Cohort 2 will have one study visit in which blood and urine samples will be collected

Sample Collection

Cohort 1 – Multiple PK visits are scheduled where paired blood and urine specimen are obtained simultaneously. Blood samples (1 to 1.5 mL each) and urine samples for evaluation of TFV levels will be collected daily between 9am and 11am (± 1 hour). The first paired samples (blood/urine) must be obtained 1-3 hours post dose of TAF/FTC and will be collected under direct observation in the clinic (Day 6 for cohort 1a, Day 0 for cohort 1b). Subsequently samples will be obtained daily between 8am and 12pm (D7-D15 for cohort 1a, D1-D6 for cohort 1b). Flexibility is allowed in collecting the post-dose samples so that a range of sample times can be obtained.

Cohort 2 – A single PK visit is scheduled where blood and urine specimens are obtained simultaneously. A blood sample (1 to 1.5mL each) and a morning urine sample for evaluation of TFV levels will be collected randomly. To obtain a standardized urine sample across study subjects, participants will be instructed to urinate once after waking up, and then to come to the study site prior to the second urination of the morning, which will be collected upon arrival prior to taking their TAF/FTC-based medication for that day. If this protocol is not followed, the subject will be asked to return on the following day. To enhance the quality of PK data collection, subjects will be asked to complete a diary card with the date and the time when they administered their TAF/FTC-based antiretroviral regimen prior to the scheduled PK visit.

Sample Processing for all Cohorts:

All blood samples will be collected in vacutainers (BD Diagnostics) containing 8.55 mg K3EDTA, kept on ice, and centrifuged at 2,600 rpm at 4°C for 15 min. The resulting plasma will be aliquoted and stored at −70°C until shipping. Samples will be shipped overnight on dry ice. All urine samples will be collected and a 10 mL sample will be aliquoted and stored at −70°C until

shipping. Samples will be shipped overnight on dry ice.

We will notify Dr. Ganesh Moorthy by e-mail when samples are shipped so that we can track, receive, and confirm the receipt of samples. The shipping address at CHOP is listed below:

Shipping address
Ganesh S. Moorthy, Ph.D.
Division of Clinical Pharmacology & Therapeutics The Children's Hospital of Philadelphia
4200 Colket Translational Research Building
3501 Civic Center Blvd,
Philadelphia, PA 19104
Phone: 215.590.0142
E-mail: moorthyg@email.chop.edu

Safety and Adverse Events

Safety and Compliance Monitoring

For Cohort 1, a comprehensive chemistry panel, pregnancy test, and hepatitis B testing will be collected as per the diagram above.

Medical Monitoring

Investigator only:

- Due to the low-risk nature and short time frame of the study, it should not be necessary to utilize an external data safety and monitoring board (DSMB). Medical monitoring will be conducted internally by the investigator and investigator team. Adverse events will be reported to the IRB in a timely fashion as well as updates on screening, enrollment, and study completion.

Definitions of Adverse Events

Adverse events (AEs), serious adverse events (SAEs), and unexpected adverse events are defined as per the International Conference on Harmonisation.

Assessment of Adverse Events

All AEs will be assessed by the Principal Investigator or qualified designee and recorded on the Case Reports Form (CRF). The AE entry should indicate whether or not the AE was serious, the start date (AE onset), the stop date (date of AE resolution), whether or not the AE was related to a study procedure, the action taken with investigational medicinal product due to the AE, and the severity of the AE. The Investigator is responsible for final review and confirmation of accuracy of events, relationship and severity confirmed by the signature on the CRF. The relationship to the study procedure should be assessed using clinical judgment and the following considerations:

- No: Evidence exists that the adverse event has an etiology other than the study procedure. For SAEs, an alternative causality must be provided (e.g., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

- Yes: A temporal relationship exists between the AE onset and a study procedure that cannot be readily explained by the subject's clinical state or concomitant therapies. Furthermore, the AE appears with some degree of certainty to be related, based on the known risks of study procedures

Reporting Procedures

We will use the AIDS Clinical Trials Group (ACTG) criteria for Adverse Events to report and grade all adverse events, whether or not they are related to the medications used in this study.

Post-study Reporting Requirements

All AEs and SAEs including deaths, regardless of cause or relationship, must be reported for subjects on study (including any protocol-required post-treatment follow-up). Investigators are not obligated to actively seek AEs or SAEs beyond the follow-up period for subjects. However, if the Investigator learns of any SAEs that occur after study participation and the event is deemed relevant to the use of investigational medicinal products, she should promptly document and report the event to the IRBs.

Limitations

It is possible that the urine assay will not be able to detect TFV in the urine of subjects taking TAF-based regimens. Although we believe this will be unlikely after discussion with the Co-Investigator of this study (Dr. Zuppa) and additional pharmacology experts, we will carry out cohort 1a first and all samples will be analyzed prior to proceeding with cohort 1b; if no TFV is detected in cohort 1a in which participants will have reached steady state, then we will not move on to cohort 1b. This pilot study also does not address the performance of the urine test under different pathologic conditions (hematuria, proteinuria, infection) or different volume states (excessive hydration, dehydration). While we do not anticipate that these conditions will affect the urinary concentration of TFV as we are measuring on a log scale (i.e. a subject would have to drink an excessive volume of water to bring the concentration of urine TFV from > 1000 ng/mL down to > 100 ng/mL), we will collect urine studies (urinalysis, specific gravity, creatinine concentration) to normalize urine TFV results using methodology to correct urine studies for illicit drug levels (Cone EJ, et al. Normalization of urinary drug concentrations with specific gravity and creatinine. *J Anal Toxicol.* 2009).

STATISTICAL PLAN

Sample Size Justification:
A sample size of 10 subjects per cohort was calculated in line with the generally accepted sample size range (4-12 subjects) for descriptive pharmacokinetics studies, as this sample size is expected to be sufficient to characterize the basic PK parameters for the parent drug in urine and plasma (Ken Thummel, “PK Studies: Design & Considerations;” available at http://courses.washington.edu/pharm309/Thummel.pdf). A population-level PK trial, which would require a much larger sample size, is beyond the scope of this study.

Statistical Analysis:
Cohort 1) We will look at the time course of TFV decay in the urine in subjects given seven daily doses of TAF/FTC (Aim 1a) and in subjects given one dose of TAF/FTC (Aim 1b) to assess the differences in patients with different adherence patterns. We will calculate the approximate half-life of tenofovir (TFV) in the urine by determining the mean number of days that the urine remains positive for TFV after TAF/FTC dosing. We will provide mean and standard deviations of the estimates. Sensitivity and specificity will be calculated over a variety of cut-points for urine TFV concentration to determine which value can be used to indicate recent adherence (in the last 1-2 days). This analysis will allow us to determine the significance of a negative value (i.e. if TFV is reliably cleared from the urine in all subjects by 5 days, then a value of 0 ng/mL can be interpreted by a clinician as suggestive that a patient has not taken medication for at least 5 days). We will compare these data to historical data in subjects given TDF/FTC, with regard to half-life in the urine, calculation of a look-back period, and the concentration of urine TFV associated with protection.

Based on the pharmacokinetics of TAF, we hypothesize that comparable cut-offs indicative of no/intermittent/recent adherence in patients on TAF-based regimens will be approximately 1 log (or 10-fold) lower. Therefore, the concentration of TFV in the urine associated with recent adherence of TAF/FTC (last 24-48 hours) is likely to be closer to 100 ng/mL, compared to 1000 ng/mL seen in the TDF/FTC validation study. As the lower limit of detection (LOD) is 5 ng/mL, we should easily have the capability to detect therapeutic dosing of TAF/FTC. We will calculate the approximate half-life of tenofovir (TFV) in the urine by determining

the mean number of days that the urine remains positive for TFV after TAF/FTC dosing. We will provide mean and standard deviations of the estimates. Based on our previous data, and under the assumption that subjects given TAF/FTC will be starting with urine TFV concentrations 10-fold less than subjects previously given TDF/FTC, we hypothesize that TFV will be present in the urine of these subjects for 3-5 days and in the plasma for 0-1 days in subjects given one dose of TAF/FTC. Subjects in cohort 1a who are at or close to steady state prior to urine testing may excrete slightly higher concentrations of the drug, and we anticipate that urine will remain positive for 1-2 days longer in these subjects. Sensitivity and specificity will be calculated over a variety of cut-points for urine TFV concentration to determine which value can be used to indicate recent adherence (in the last 1-2 days). This analysis will allow us to determine the significance of a negative value (i.e. if TFV is reliably cleared from the urine in all subjects by 5 days, then a value of 0 ng/mL can be interpreted by a clinician as suggestive that a patient has not taken medication for at least 5 days).

Cohort 2)

We would like to determine how urine TFV concentrations in HIV-positive subjects taking a TAF-based regimen compare to those taking TDF-based regimens. We previously showed in ten HIV patients taking daily TDF-based antiretroviral therapy regimens that detection of urine TFV corresponds to plasma TFV concentrations within a 24 hour dosing window. Since TAF concentrates more highly in the intracellular space and less in the plasma space, resulting in approximately 10% of the plasma TFV concentrations compared to TDF, we anticipate that both plasma and urine TFV concentrations will likewise be approximately 10% of the values in TDF patients (i.e. 1 log lower) assessed under the same conditions.

We will analyze these data visually and both qualitatively (is there detectable TFV in the urine or plasma or not?) and quantitatively (what is the mean concentration of TFV detected in spot urine and plasma collections?). For the quantitative analysis we will calculate the Pearson correlation coefficient and standard deviation (or Spearman if the variables are not normally distributed) between urine and blood TFV concentrations. For the qualitative analysis (presence or not of TFV) we will create 2X2 contingency tables and calculate chi-squares or Fisher exact tests. We will compare these results to historical data done under the same conditions in subjects taking fixed-dose combination pills containing TDF/FTC using Pearson correlation coefficients.

We hypothesize that TFV will be detectable in urine in all or most patients in this cohort of presumably adherent patients since even a 1-log reduction in TFV concentration relative to measurements in patients on TDF-based regimens should still result in detectable urine concentrations. We likewise hypothesize that plasma TFV concentrations will be approximately 1-log lower in this cohort compared to the historical controls, and may not be detectable in all subjects. We also anticipate that the qualitative correlation between urine TFV concentrations in this cohort of subjects compared to a historical cohort of subjects taking TDF-based regimens will be robust. This aim will provide important information on the quantitative vs. qualitative role for urine TFV testing in subjects on TAF, and provide a preliminary comparison of the difference in urine TFV concentrations in patients on TAF- versus TDF-based regimens in HIV-positive patients taking antiretroviral therapy.

DATA HANDLING AND RECORD KEEPING

Confidentiality and Security

Confidentiality of the research records is maintained carefully. Subjects will be assigned a specific Patient Identification number (PID) upon entry into the study, after which all medical information is referred to by this number. Study records are maintained in a secure location on site. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. Publication of data will not identify subjects by name. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the NIH or other funding agency and the OHRP.

Training

This study includes research with human subjects. IRB approval of the protocol and the informed consent will be obtained from both the University of Pennsylvania IRB and the Philadelphia FIGHT IRB. Dr. Helen Koenig and Dr. Athena Zuppa maintain up-to-date training in Protection of Human Subjects and Good Clinical Practice (GCP), and all clinical and demographic information will be protected as per HIPAA guidelines. All research assistants will have GCP and human protection training as well as study-specific training.

Case Report Forms and Source Documents

CRFs will be provided for each subject. Subjects must not be identified by name on any CRFs. Subjects will be identified by the PID provided by Philadelphia FIGHT. Instructions concerning the recording of study data on CRFs will be provided by the Principal Investigator (PI), and the PI and research assistants will monitor on a real-time basis the individual subject records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The PI will also be responsible for ensuring that regulatory requirements are being followed. The investigator will make study documents (e.g., consent forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the NIH or other funding agency, and the Office for Human Research Protections (OHRP) of the University of Pennsylvania.

STUDY MONITORING, AUDITING, AND INSPECTING

Study Monitoring Plan

This study will be monitored internally. Samples from cohort 1a will be completely analyzed and, if TFV is not able to be detected in 1a specimens, we will not proceed to cohort 1b.

Auditing and Inspecting

This protocol and the informed consent documents and any subsequent modifications will be reviewed and approved by the University of Pennsylvania and Philadelphia FIGHT IRBs. A signed consent form will be obtained from the subject describing the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject and the original will be kept in the subject's record.

Study Discontinuation

The study may be discontinued at any time by the IRBs, the NIH or other agencies as part of their duties to ensure that research subjects are protected.

STUDY ADMINISTRATION

Organization and Participating Centers

The study will be conducted at any of the health centers at Philadelphia FIGHT (an AIDS service organization affiliated with the University of Pennsylvania): the adult HIV clinic (Jonathan Lax Treatment Center), the adult primary care clinic (John Bell Health Center) and the youth drop-in center (Youth Health Empowerment Project, or Y-HEP). Urine and blood samples will be collected at Philadelphia FIGHT and delivered to the

laboratory at the Children's Hospital of Philadelphia, and all other safety and screening labs will be run by Labcorp.

Funding Source and Conflicts of Interest

This grant proposal will be funded through Gilead Sciences, Inc. Additional salary support and indirect costs will be covered by Philadelphia FIGHT.

Collaboration with other scientists or research institutions

We will collaborate with the Pharmacology Research Unit at the Children's Hospital of Philadelphia (CHOP) to re-validate the urine tenofovir assay for patients taking TAF-based regimens. Subject recruitment and sample collection will be done at Philadelphia FIGHT, and urine specimens will be provided to CHOP for analysis.

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